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Award Number: W81XWH-09-1-0072

TITLE: Canonical Wnt Signaling as a Specific Marker of Normal and Tumorigenic Mammary Stem Cells

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REPORT DATE: February 2013

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
February 2013	Final	1 February 2009 – 31 January 2013

4. TITLE AND SUBTITLE
Canonical Wnt Signaling as a Specific Marker of Normal and Tumorigenic Mammary Stem Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0072

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Van Andel Research Institiute
Grand Rapids, MI 49503

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have determined that the Wnt/B-catenin signaling pathway is active within a small subset of cells within the gland. This small subset is entirely contained within the previously identified population that has the ability to repopulate a cleared mammary fat pad. Thus, activation of canonical WNT signaling may be very specific marker for mammary stem cells and be a target for transformation that results in the formation of aggressive mammary tumors.

15. SUBJECT TERMS- Breast cancer stem cells, Wnt signaling, caononical Wnt signaling, B-catenin, normal stem cells, adult stem cells.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU		USAMRMC

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Introduction

The overarching goal of this proposal was to test the hypothesis **that activated canonical Wnt signaling identifies mammary stem cells and mammary tumor cells with cancer stem cell properties.** Our objective is to determine whether this is the case by transplantation experiments of normal mammary cells and mammary tumor cells that exhibit activated canonical Wnt signaling.

Activation of the Wnt pathway is associated with abnormal mouse mammary development, tumorigenesis, and human breast cancer. In addition, increasing evidence suggests that tumors arise from either normal stem or progenitor cells through the deregulation of self-renewal processes [1]. The existence of mammary stem cells was established by transplantation experiments in mice. For example, a functioning ductal tree can be regenerated using very few transplanted mammary cells carrying CD24 and CD49f cell surface markers: although only 1 in 20 to 90 $CD24^{+}/CD49f^{++}$ mammary cells is a true stem cell [2]. We have found that activated Wnt/β-catenin signaling is restricted to a sub-population of mammary tumor cells that are $CD24^{+}/CD49^{++}$. Since Wnt pathway activation occurs in human breast cancer and is required for proliferation of various other stem cell compartments, addressing how Wnt signaling promotes mammary stem cell renewal and the role this plays in breast cancer will ultimately lead to more effective treatments for breast cancer.

Body

Note: We have organized this section to include each of the eight tasks outlined in the "Statement of Work" (SOW) followed by a summary of the work done toward completing each of these tasks. The original tasks are listed with bold type.

Aim 1: To determine whether canonical Wnt signaling through β-catenin is associated with specific markers of mammary stem cell activity.

Task 1. Collect mammary glands from *BATgal* transgenic female mice [3] at different stages of mammary development: embryonic (day 12.5 and 18.5), newborn (7 day-old), juvenile (5 week-old), adult virgin (12 week-old) and pregnant (day 6.5, 12.5 and 18.5). Mammary glands from four mice per time point will be isolated. A total number of 32 female mice will be included in this part of the study. One abdominal mammary gland will be used for whole mount LacZ staining to determine the expression pattern of β-galactosidase (β-gal). *BATgal* Wnt reporter mice exhibit expression of β-gal in cells that have activated Wnt/β-catenin signaling. Hence, β-gal expression can be used as a marker for canonical Wnt signaling. Mammary whole mount LacZ staining will be performed at the time the mammary gland is isolated.

The other abdominal and both inguinal glands will be processed to single cell mammary cell suspensions. Any lymph nodes will be removed. The isolated glands will be chopped into small pieces using scissors and then dissociated into single cells using a standard protocol that includes digestion with collagenase/hyaluronidase followed by trypsin, dispase/DNase, and ammonium chloride. The

cell suspension will be filtered and then frozen and stored in liquid nitrogen until required for further analysis.

We will similarly collect tissues and cells from *BATgal;MMTV-Wnt1*-induced mammary hyperplasia and tumors [4]. *BATgal;MMTV-Wnt1* mammary glands and mammary tumor tissues will be analyzed for β-gal expression using LacZ and single cell suspension will be isolated, frozen and stored as well. We will collect mammary glands from eight *BATgal;MMTV-Wnt1* females and tumor tissues and cells from eight tumor-bearing mice. Collection of mammary whole mounts and tumor tissues, LacZ staining and isolation of single mammary cell and tumor cell suspensions will take approximately one year. A total number of 48 female mice will be included.

In previous reports we have clearly showed that cells with activated canonical Wnt signaling are present within the mammary epithelium starting at embryonic day 12.5 through adulthood as determined by LacZ staining. In adult glands Wnt responsive cells are few in number and are located in the basal cell layer where mammary stem cells are thought to reside. There is a significant expansion of cells undergoing canonical Wnt signaling in *MMTV-Wnt1* pre-neoplastic mammary glands compared to controls. The expansion of LacZ positive cells is proportional to the increase in ductal hyperplasia within the Wnt1 transgenic model. This data shows that Wnt-responsive cells reside in the mammary stem cell niche as determined by Lac Z staining of *BATgal* reporter animals.

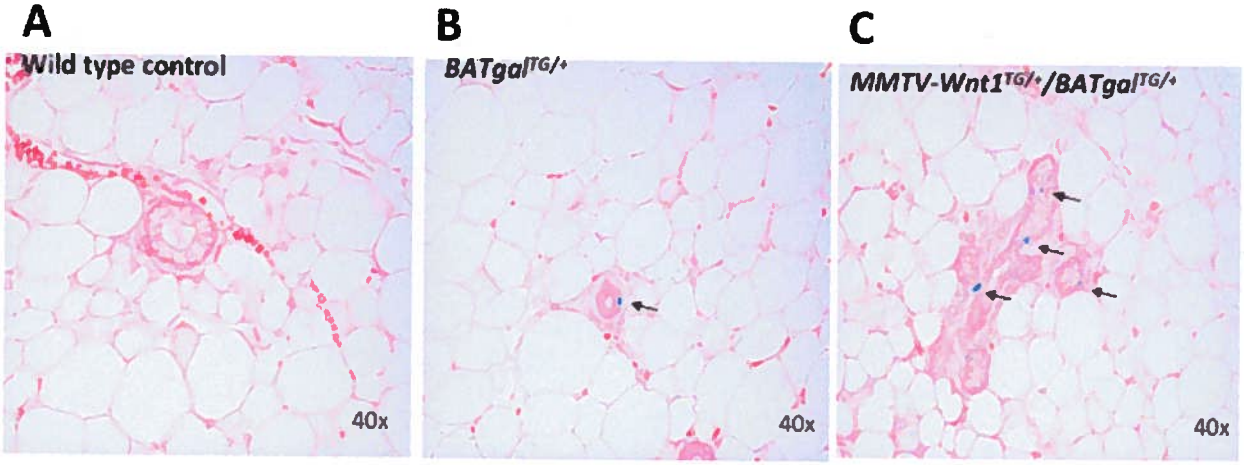

Figure 1: Increased number of Wnt responsive cells in MMTV-Wnt1 mammary glands. LacZ stained mammary glands from adult (A) control (B) BATgal transgenic (C) MMTV-Wnt1/BATgal double transgenic animals. BATgal positive cells are few in number and reside in the basal cell layer. There is an increase in the number of BATgal positive cells in MMTV-Wnt1 transgenic glands.

<u>Task 2.</u> We will analyze single mammary cell suspension by FACS using cell surface markers and the β-gal substrate DDAOG. We will first label single cell suspensions with CD31, CD45, and TER119. These antibodies mark endothelial and hematopoietic cells (Lin$^+$) and will be excluded from further analysis. The remaining Lin$^-$ mammary cells will be labeled with CD24 and CD49f which marks the stem cell enriched cell population. We will identify cells with canonical Wnt signaling by staining for β-gal reporter gene activity using the β-gal substrate DDAOG. DDAOG is detectable in far red. FACS analysis

of Wnt canonical signaling activity at different time points of mammary development and of Wnt1-induced mammary tumors will take approximately two months to complete.

Using FDG (fluorescein di-β-D-galactopyranoside) as a substrate for β-gal activity our flow cytometry data suggests there is a 2-fold increase in the number of FDG$^+$ MEC's in *BATgal* animals compared to controls which further supports our LacZ staining (Figure 2). We are currently characterizing the expression levels of CD24 and CD49f within FDG+ cells to determine if this population of cells is enriched for stem cell activity. Flow cytometry will determine the percentage of FDG$^+$ cells within pre-neoplastic *BATgal/MMTV-Wnt1* MEC's. After many attempts to look at FDG$^+$ cells in *BATgal/MMTV-Wnt1* tumors we discovered tumors from these animals are hypoxic, as determined by HIF1α immunohistochemical staining, and in normoxic conditions lead to rapid cell death.

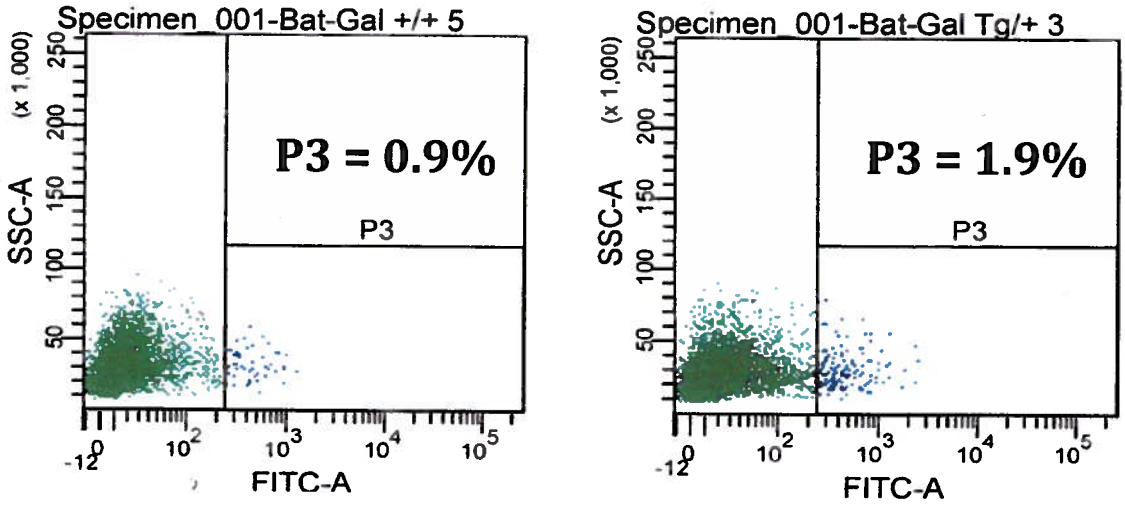

Figure 2: Detection of Wnt responsive cells by FDG. MEC's were collected from wild type and *BATgal* transgenic animals and incubated with FDG (fluorescein di-β-D-galactopyranoside) a substrate for β-galactosidase. Dot plots indicate the percentage of living FDG positive MEC's (P3 population) for both wild type and *BATgal* transgenic animals as determined by flow cytometry.

<u>Task 3.</u> Determine the hormone receptor status of mammary cells with activated Wnt signaling. We will isolate cells with and without active canonical Wnt signaling onto microscopic slides and perform immunohistochemistry for the estrogen and the progesterone receptors using commercially available antibodies from Cell Signaling Technologies. These experiments will be performed in adjunction to the FACS experiments described in Task 2 and will take two months to complete.

We have optimized antibodies against the estrogen and the progesterone receptor for use on the Ventana automated staining system. We have stained sections of *BATgal/MMTV-Wnt1* tumor tissue and found that these tumors express estrogen receptor. Previously we have using LacZ staining to determine β-gal activity within the mammary gland, which renders the tissue unusable for immunohistochemistry. We have been unsuccessful in co-staining β-galactosidase, ER and PR to determine the hormone receptor status of β-gal positive cells. The commercially available antibodies for β-gal are not optimal

for IHC in the mammary gland. We are currently looking into new approaches to show the expression of these hormone receptors on Wnt responsive cells. We will go with our initial approach of collecting FDG^+ cells and perform immunocytochemistry to look for expression of hormone receptors.

Aim 2. To determine the stem cell activity of mammary cells with activated Wnt/β-catenin signaling.

Task 1. We will test whether Wnt responsive cells possess the majority of the stem cell activity by limiting dilution transplantation experiments. $Lin^-/CD24^+/CD49f^{++}/DDAOG^+$ (test) and $Lin^-/CD24^+/CD49f^{++}/DDAOG^-$ (control) mammary cells from adult virgin *BATgal* transgenic females will be isolated by FACS as described under Aim 1. Cells with canonical Wnt signaling will be identified as $DDAOG^+$. Based on our previous experience, we will need approximately 8 *BATgal* transgenic female mice to get enough sorted mammary cells for the transplantation experiments.

We are currently working with our Flow Cytometry Core to sort $Lin^-/CD24^+/CD49f^{++}/FDG^+$ (test) and $Lin^-/CD24^+/CD49f^{++}/FDG^-$ (control) cells for transplantation into the cleared fat pad of NSG mice. Our Flow Core purchased two new cytometers that are able to sort cells in a sterile environment. Our facility purchased the CyFlow Space, made by Partec, and the MoFlo Astrios, made by Beckman Coulter. Both of these pieces of equipment are in house and we are currently working with the Flow Manager to optimize these experiments.

Task 2. We will inject 10, 50, 100, and 1000 test and control cells into the cleared fat pads of 3-week-old *Rag2*-deficient females. We will inject 10 $Rag2^{-/-}$ females with 10 test cells into one abdominal mammary fat pad and 10 control cells into the contralateral fat pad, 30 $Rag2^{-/-}$ females with 50 test and 50 control cells, 10 $Rag2^{-/-}$ females with 100 test and 100 control cells, and 10 $Rag2^{-/-}$ females with 1000 test and 1000 control cells. We will isolate the host fat pads after six weeks and determine ductal out-growth. We estimated the number of animals to be used for this via the following rationale. Limiting dilution experiments have found that on average 1/64 $Lin^-/CD24^+/CD49f^{++}$cells is a mammary stem cell. Hence, transplanting 10 $Rag2^{-/-}$ females with 10 test and control cells should result in around 3 out-growths with control cells. If we assume that the presence of canonical Wnt signaling will be associated with a three-fold enrichment of stem cells, we would predict that 90% (9 out of 10) of the transplants with test cells will repopulate the cleared mammary fat pad. Power analysis (powered to 80% for a Type I error of 0.05) suggests that it will be necessary to use 10 animals in each group. If we assume that 75% of cleared mammary fat pads will be repopulated via injection of 50 control cells and all will be repopulated with test cells (assuming at least a three fold enrichment), power calcuations (again powered to 80% with a Type I error of 0.05) suggest we will need to use at least 27 $Rag2^{-/-}$ females for each group. We predict that virtually all cleared fat pads will be repopulated when either 100 or 1000 control cells are injected. Therefore, we will inject 5 $Rag2^{-/-}$ females with test and control cells at 100 cells/gland and 1000 cells/gland and evaluate these as positive controls for these experiments. These experiments will take one year to complete and will include 60 $Rag2^{-/-}$ females.

As mentioned in our previous report we have switched our recipient animal model from *Rag2*-deficient females to NOD/SCID/IL-2 receptor gamma females (NSG) as transplantation recipients. NSG mice have recently been shown in a variety of settings to be a superior and more efficient choice for these types of studies (for example see [6]). We have acquired NSG and are currently expanding the colony. We have a sufficient number of animals to perform the transplantation experiments once cells have been sorted.

As stated in task 1 of this aim once the FACS core is up and running we will be able to sort cells for transplantation experiments.

Aim 3. To determine the tumorigenicity of *MMTV-Wnt1* tumor cells with activated Wnt/β-catenin signaling.

<u>Task 1</u>. We will test whether β-gal$^+$ cells isolated from *BATgal/MMTV-Wnt1* mammary tumors specifically confer tumorigenesis in a transplantation model. When tumors develop in *BATgal/MMTV-Wnt1* females we will dissect the tumors and isolate single cell suspensions using the automated mechanical disaggregation system Medimachine from Becton Dickinson. The sorted tumor cells will be frozen and stored in liquid nitrogen until needed for further analysis.

We have taken another approach to understanding how changes in Wnt/β-catenin signaling affect the tumorigenic potential of *MMTV-Wnt1* transgenic cells. We generated a mouse line in which β-catenin is conditionally deleted in the mammary epithelium of *MMTV-Wnt1* transgenic animals. Using MMTV-cre (line A) animals in which cre recombinase is expressed in the mammary epithelium, we created MMTV-cre$^{Tg/+}$-β-catenin $^{flox/flox}$;MMTV-Wnt1$^{TG/+}$ mice. We hypothesized that the loss of β-catenin in Wnt1 driven tumors would lead to a delay in tumorigenesis.

First we wanted to determine the expression of our MMTV-cre expression within the mammary gland. To do this we crossed our MMTV-cre mice to the mT/mG Cre reporter mouse. The mTmG reporter mice possess *loxP* sites on either side of a membrane-targeted tdTomato (mT) cassette. In cells in which cre is expressing, the mT cassette is deleted allowing expression of the membrane-targeted EGFP (mG) cassette located downstream. We analyzed MMTV-cre-mT/mG glands by confocal microscopy and observed cre recombination is approximately 98% of the mammary epithelium (Figure 3).

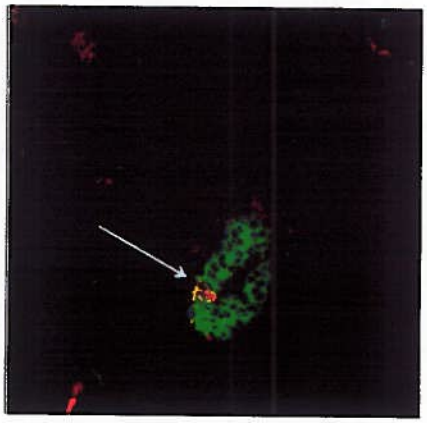

Figure 3: Cre recombination in mammary epithelium. Using the Cre reporter mT/mG mouse we can detect Cre recombination in mammary epithelium based on expression of GFP. The arrow shows the lack of GFP expression in a single cell within the mammary epithelium of a MMTV-cre-mT/mG gland.

Next we explored how the loss of β-catenin in the mammary epithelium impacts glandular development. We found ductal abnormalities; however, the phenotype was not as severe as expected. Approximately 5% of the mammary epithelial cells contain normal levels of β-catenin. We believe these 'normal cells' are able to form the impaired ductal structure that exists in β-catenin ^{cKO} mice (Figure 4).

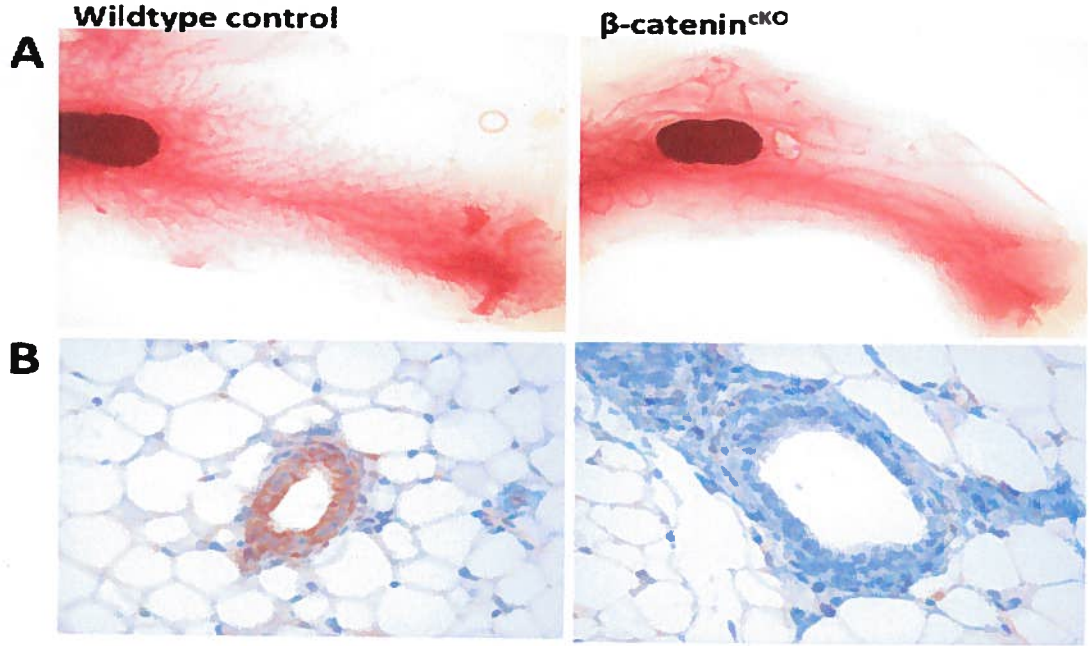

Figure 4: β-catenin deficient mammary glands have developmental defects. Mammary glands were collected from 11 week old wild type and β-catenin conditional knockout animals. Shown in (A) are whole mount images of Carmine staining and (B) immunohistochemical staining for β-catenin.

When we cross the MMTV-cre$^{Tg/+}$-β-catenin $^{flox/flox}$ mice to MMTV-Wnt1 $^{Tg/+}$ animals we see no change in

tumor onset as compared to MMTV-Wnt1 $^{Tg/+}$ animals alone. Potentially this result is due to the incomplete recombination of β-catenin in a small portion of the epithelium. The cells that have normal β-catenin levels appear to transduce the Wnt1 signal leading to tumorigenesis.

Next we investigated whether elimination of Wnt/β-catenin signaling at the receptor interface would create the same phenotype as the β-catenin ^{cKO} mice. Our past work using knockout models showed that loss of either Lrp5 or Lrp6 alone significantly delayed mammary development, with Lrp6 heterozygous knockout animals demonstrating the more severe phenotype. Since homozygous Lrp6 knockout animals are embryonic lethal, we utilized conditional mouse models. We have generated conditional models of both Lrp5 and Lrp6 using the same MMTV-cre transgenic mouse model mentioned above. Our initial experiments focused on how loss of Lrp5, Lrp6, and both receptors impacts the developing mammary gland. Typically during puberty extensive ductal elongation occurs which fills the entire fat pad. We found significant delays in ductal branching in either Lrp5 or Lrp6 adult glands (Figure 5). Mice lacking both Lrp5 and Lrp6 genes (Lrp5^{cKO}Lrp6^{cKO}) have a very primitive ductal structure with no evidence of branching at 11 weeks of age compared to controls with a penetrance of 100%.

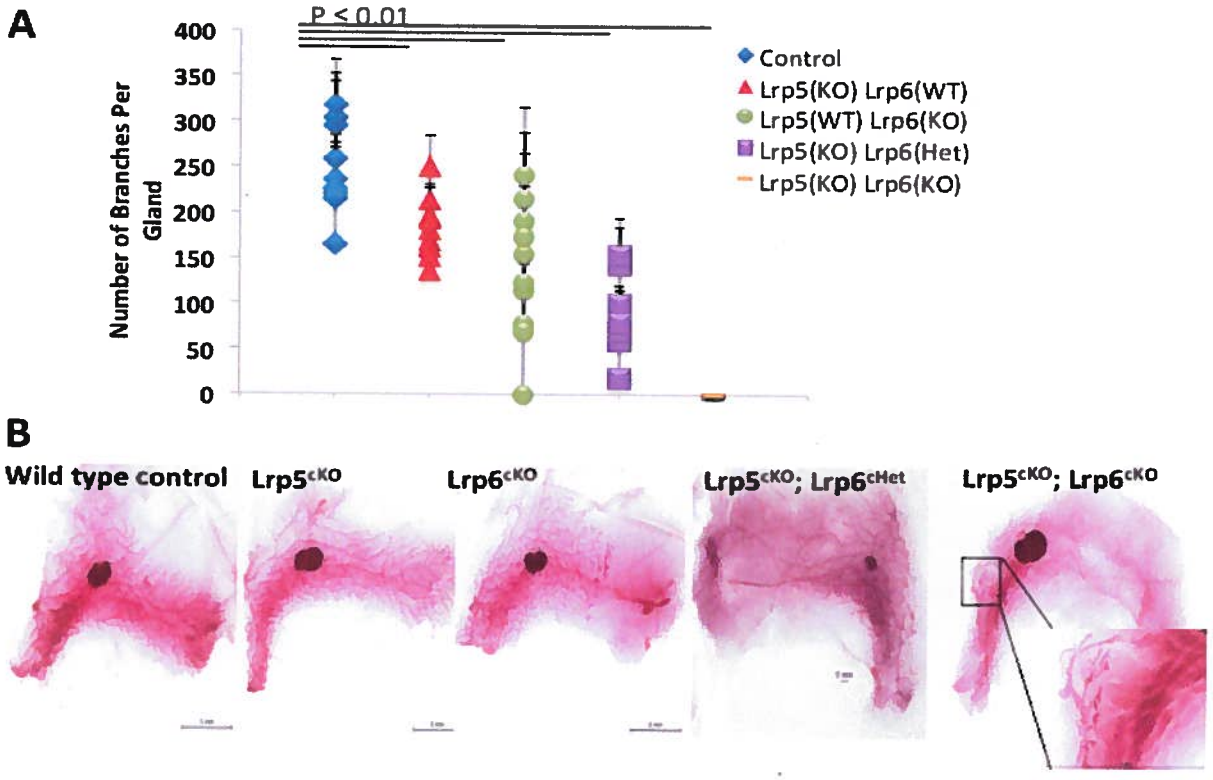

Figure 5. Lrp5 and Lrp6 are necessary for ductal branching. The number of ductal branches were counted in 11 week old Lrp5^{cKO}, Lrp6^{cKO}, and Lrp5^{cKO};Lrp6^{cKO} glands (A). There was a statistical decrease in the number of branches in all lines. Carmine images (B) show ductal structure of each genotype.

H&E staining of sections from Lrp5c^{KO}; Lrp6^{cKO} glands showed normal basal/luminal architecture similar

to controls. We then wanted to determine the expression of β-catenin in the ducts of these glands. There appeared to be no significant difference in the expression of β-catenin in Lrp5^{cKO} or Lrp6^{cKO} glands alone. We observed decreased nuclear β-catenin in Lrp5c^{KO}; Lrp6^{cKO} glands (Figure 6). Interestingly there appears to be an increase of cytoplasmic β-catenin in the adipocytes of the sections, which requires further exploration.

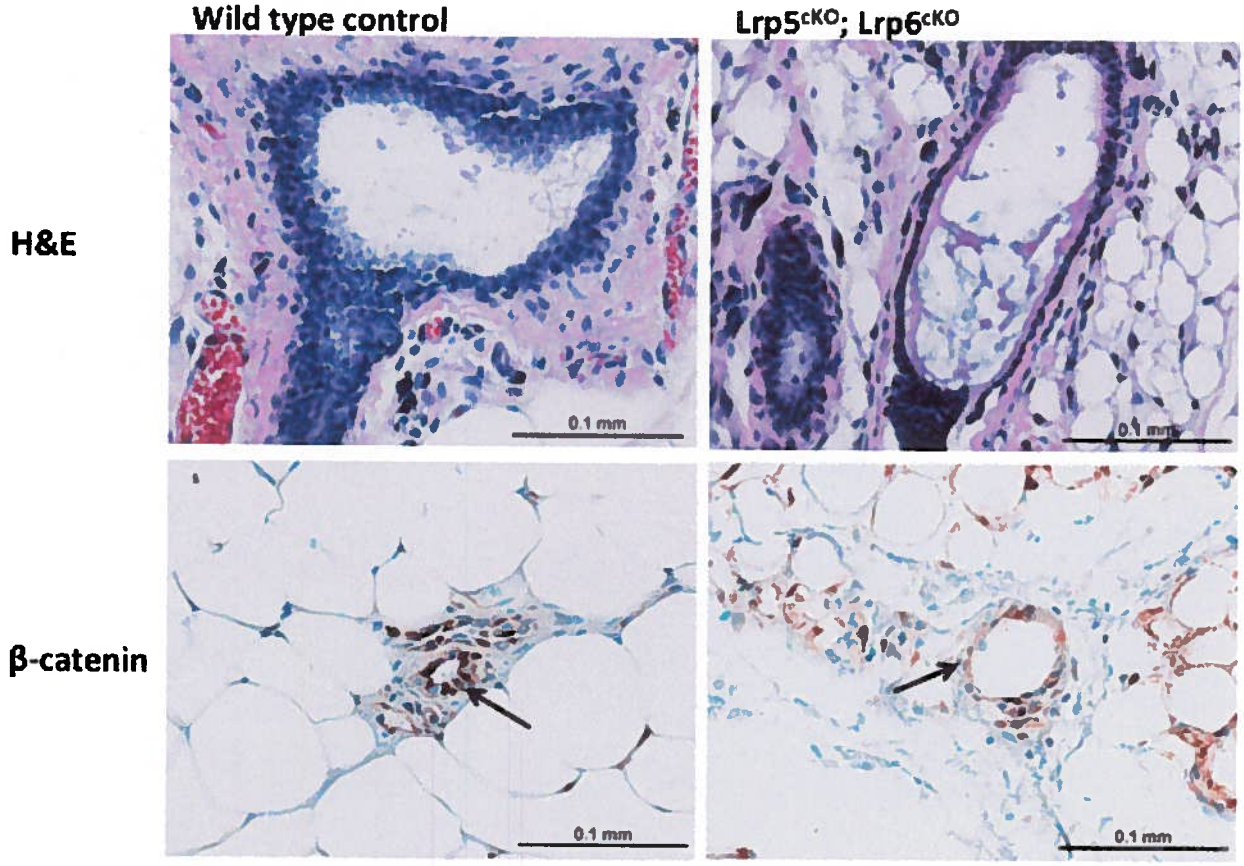

Figure 6. Nuclear β-catenin is decreased in ductal epithelium of Lrp5^{cKO}; Lrp6^{cKO} glands. Ductal morphology of Lrp5^{cKO}; Lrp6^{cKO}, as determined by H&E staining, shows no difference compared to control. Immunohistochemistry for β-catenin on Lrp5^{cKO}; Lrp6^{cKO} sections shows decreased nuclear staining as compared to controls.

Next we wanted to know if hormone signaling during pregnancy impacts the ductal abnormalities seen in Lrp5/Lrp6 deficient glands. We collected glands from E12.5 and E17.5 pregnant nulliparous females. In Lrp5^{cKO}; Lrp6^{cKO} animals we noticed no ductal elongation, side branching, and no presence of alveologenesis unlike control glands (Figure 7). Lrp5^{cKO}; Lrp6^{cKO} animals were able to carry embryos to term but were unable to nurse their young due to lack of milk production. We noticed our results were very similar to studies looking at the loss of epithelial ERα. ERα deficient mammary glands develop normally until puberty where after no development occurs even during pregnancy [8]. One thing we did notice in Lrp5/Lrp6 deficient glands was the presence of terminal end buds during pregnancy. This result made us question how multiple pregnancies would impact the glands of Lrp5/Lrp6 deficient animals. Females undergoing their second pregnancy show signs of ductal elongation, increased side

branching and the start of alveologenesis. However, the recovery is still not enough to nurse their young. Our results suggest that Wnt/β-catenin signaling plays an important role in establishing the mammary epithelium during development and also plays a role in pregnancy-induced mammary proliferation. We are currently looking at the expression of ERα in the glands of Lrp5/Lrp6 deficient animals to get a better idea of the significance of our phenotype.

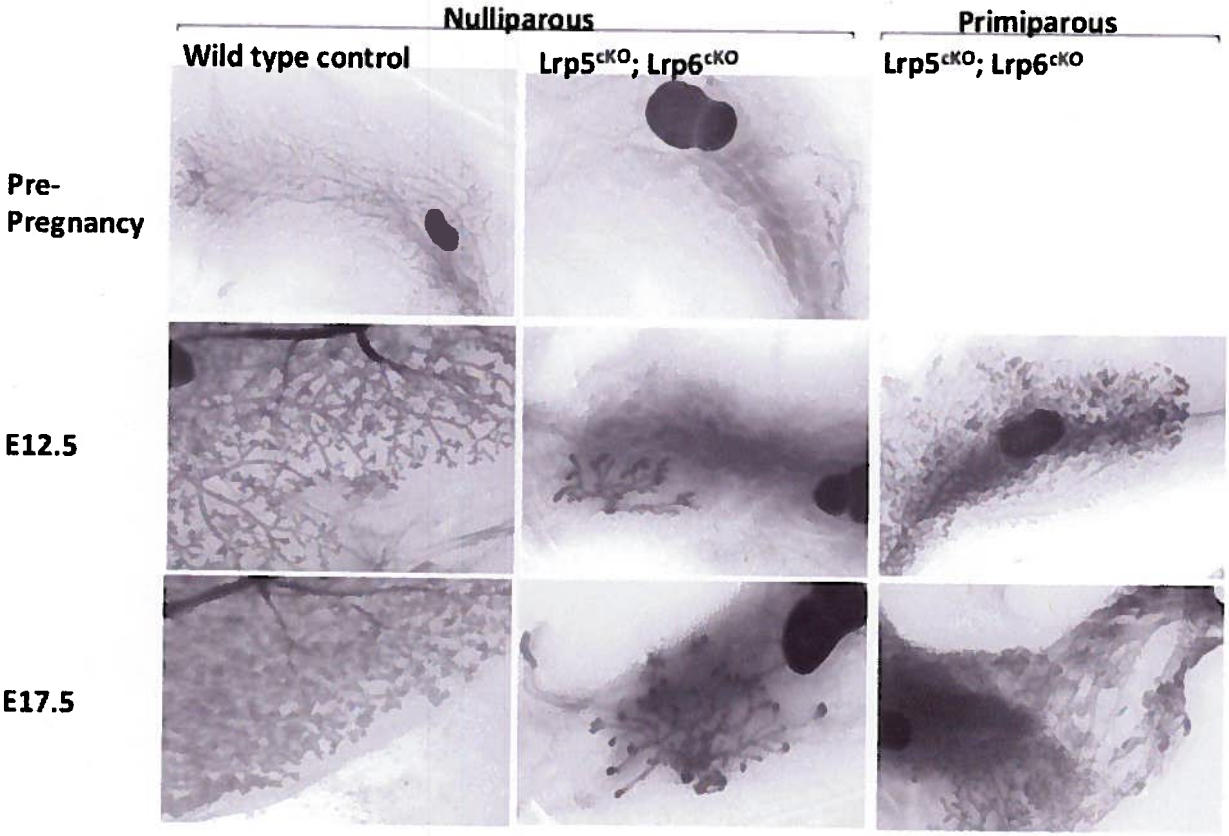

Figure 7. Lrp5 and Lrp6 play a role in pregnancy-induced proliferation. Analysis of carmine stained glands from nulliparous E12.5 and E17.5 $Lrp5^{cKO}$; $Lrp6^{cKO}$ females show no ductal proliferation. Mammary glands from primiparous $Lrp5^{cKO}$; $Lrp6^{cKO}$ females show some recovery during pregnancy.

Task 2. Isolation of Lin$^-$ tumor cells with (DDAOG$^+$) and without (DDAOG$^-$) canonical Wnt signaling will be achieved using FACS as described in Aim 1. Limiting dilutions of 10, 100, 1000, 50,000 of Lin$^-$/DDAOG$^+$ (test) and Lin$^-$/DDAOG$^-$ (control) tumor cells in liquid matrigel will be injected into the mammary glands of *Rag2*$^{-/-}$ immunocompromised female mice. For these experiments, we will inject mice with either test or control cells (not both into contralateral sides as in Aim 2). We used similar power calculations as described above for Aim 2, Task 2 as a base, except for doubling the amount of mice since we are injecting them with either test or control cells (not both). Therefore, we will inject 10 *Rag2*$^{-/-}$ females with 10 test cells and 10 *Rag2*$^{-/-}$ females with 10 control cells. 30 *Rag2*$^{-/-}$ females with 50 test cells and 30 *Rag2*$^{-/-}$ females with 50 control cells. We will also inject 20 *Rag2*$^{-/-}$ females (5 for each group) with either 100 or 1000 test cells and either 100 or 1000 control cells. As a further positive control, we will inject 10 mice with 100,000 unsorted cells. The *Rag2*$^{-/-}$ host mice will be monitored for tumor development. We expect to see tumor out-growths in most *Rag2*$^{-/-}$ females inoculated with 50,000 tumor cells. If canonical Wnt signaling is indeed required for cancer stem cell activity, we expect to see more tumor out-growths when limiting dilutions of DDAOG$^+$ tumor cells relative to DDAOG$^-$ cells are inoculated. Tumor growth will be measured using a caliper, and when the mice have to be sacrificed to ensure the humane treatment of animals. The tumors will then be isolated and analyzed by different means including histopathology, FACS, and gene expression profiling. These experiments will take one to two years to complete and will include approximately 8 *BATgal/MMTV-Wnt1* and 110 *Rag2*$^{-/-}$ female mice.

We will need to perform this study using Lin$^-$/CD24$^+$/CD49f^{++}/FDG$^+$ (test) and Lin$^-$/CD24$^+$/CD49f^{++}/FDG$^-$ (control) cells in limiting dilution. Our Flow Core recently purchased a new cell sorter and we will characterize and sort these cells before performing our transplantation experiments in NSG females. Currently we are optimizing the antibody dilutions and cell preparations for this experiment.

Task 3. We will determine the gene expression pattern of Wnt responsive *BATgal/MMTV-Wnt1* tumor cells. We will isolate mammary tumor cells with (DDAOG$^+$) and without (DDAOG$^-$) canonical Wnt signaling by FACS. We have found that a 5 minute exposure is enough to identify the Wnt responsive cell population by FACS. After staining with DDAOG we will fix the cells in order to preserve the relative mRNA levels and then isolate Lin$^-$/DDAOG$^+$ and Lin$^-$/DDAOG$^-$ cells by FACS. mRNA will be isolated from the cell isolates using the Trizol total RNA extraction method. We expect we will need to amplify the mRNA since our preliminary data suggest that only 0.2% of the BATgal/MMTV-Wnt1 tumor cells are Wnt responsive. For the expression profiling, we will use GeneChip Mouse Genome 430 2.0 Array from Affymetrix. We will perform these experiments in collaboration with the microarray core facility at the Van Andel Research Institute. We expect the microarray experiment to take approximately 3-4 months to complete.

We have outlined this experiment in detail with Dr. SokKean Khoo of the microarray core facility at the Van Andel Research Institute. Once we are able to properly sort and collect Lin$^-$/FDG$^+$ and Lin$^-$/FDG$^-$ MECs we will perform this analysis.

Key Research Accomplishments

1. We have determined that high β-catenin expressing cells (as measured by positive staining for BATgal) are highly enriched in mammary progenitor cells. These cells were identified by flow cytometry to detect cells that were positive for CD24 and CD49f.
2. We have established that activation of Wnt signaling expands the total number of cells in the mammary gland but does not alter the ratio of cells showing activation of canonical signaling relative to those without activation of the pathway.
3. We have shown that loss of Wnt/β-catenin signaling during mammary gland development significantly impacts mammary gland development, but Wnt/β-catenin signaling does not seem to play as significant of a role in pregnancy-induced ductal expansion.
4. We have found that mammary transplantation of cells with activated levels of canonical Wnt signaling do not appear to induce tumorigenesis.
5. We have found that mice lacking both Lrp5 and Lrp6 within the mammary epithelium have deficits in mammary development and in expansion of the mammary gland ductal network during their first pregnancy.
6. We have further demonstrated that the mammary epithelium of mice lacking both Lrp5 and Lrp6 is capable of expansion in subsequent pregnancies.

Reportable Outcomes

Work supported by this funding has been presented in the following venues

1. Translational Genomics Research Institute Annual Retreat – June 2009, Phoenix, AZ
2. Van Andel Research Institute Annual Retreat – May 2009 – Thompsonville, MI
3. Wnt Meeting – June 2009 – Washington, DC
4. Van Andel Research Institute Annual Retreat – May 2010 – Thompsonville, MI
5. Translational Genomics Research Institute Annual Retreat – September 2010, Phoenix, AZ
6. Baylor Breast Center Invited Seminar-December 2011, Houston, TX

Two trainees supported by this funding have been accepted into clinical training programs

1. Charlotta Lindvall – admission to the Grand Rapids Medical and Research Center Internal Medicine Residency Program and to the fellowship training at Dana Farber Cancer Institute.
2. Audrey Sanders – admission to the Michigan State University College of Osteopathic Medicine

Conclusion

"So-what section"

For decades, oncologists have focused on developing therapeutic approaches that shrink tumor mass. Unfortunately, while many treatments can dramatically shrink tumors initially, recurrence of the initial tumor is common. Recently, a new model has been proposed that may explain these observations. This "cancer stem cell" model postulates that, in many cases, the cell that is transformed is a cell with pluripotent ("stem-cell like") capabilities. In other words, the cell is capable of both self-renewal and producing progeny of many diverse cell types. The consequence of this is that tumors are mostly composed of cells that are descendants of the original tumor cell, but which are no longer capable of forming tumors themselves. Treatments that shrink the majority of the tumor by attacking the differentiated cells may not affect the small population of pluripotent cells that actually give rise to the tumor. Thus, the tumor recurs and eventually becomes resistant to any known treatment, leading to metastatic progression and, ultimately, the death of the patient. A great deal of experimental evidence published from many laboratories supports this model. This has tremendous implications for how we treat tumors and the types of drugs that we should try to develop to treat tumors. That is, we need to better understand the characteristics of these tumor-forming (cancer stem/progenitor) cells and develop treatments that can kill them while minimizing side effects.

We, and others, have published experiments showing that the Wnt signaling pathway is associated with normal mammary development and with mammary tumorigenesis. Recently, we have developed some very exciting data that suggests that activation of this pathway may provide a very specific marker with which to identify normal and cancer stem cells. These are based on both mouse models and on analysis of genes expressed in human breast tumors. Specifically, we have found that human mammary tumors of the basal-like class (a type thought to be the most "stem-cell like" in origin) have increased activation of this pathway. Tumors of this class do not typically respond well to currently available therapies, and therefore an urgent need exists to identify new potential targets for therapy. If our preliminary results are confirmed, it would provide an excellent target (the Wnt signaling pathway) that could be used to specifically treat this tumor type.

In this proposal, we carried out a detailed characterization of the role of Wnt signaling in normal and tumorigenic mammary stem/progenitor. Perhaps the most interesting observation we have made concerns the fact that mammary epithelium lacking Lrp5 and Lrp6 fails to develop during puberty and remains obviously defective during the first pregnancy. However, while still looking abnormal, significant ductal expansion takes place during subsequent pregnancies. This observation may have relevance to how different cell types may give rise to different types of malignancies based on their differential abilities to differentiate.

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